Essential oils of trunk, limbs, needles, and seed cones of *Pinus edulis* (Pinaceae) from Utah

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ABSTRACT

Pinus edulis Engelm. (pinyon pine) is an essential oil-bearing evergreen tree. Trunk, limb, needle, and cone samples of pinyon pine from Utah were collected, separately steam distilled, and the resulting essential oils analyzed by GC-FID and GC-MS. The different plant parts shared similar compounds but in widely varying relative percentages. In every sample α-pinene was the most abundant compound, ranging from an average of 50.3% across trunk samples to an average of 70.5% in cone samples. Other prominent compounds included sabinene, β-pinene, δ-3-carene, β-phellandrene, ethyl octanoate, longifolene, and germacrene D, each in varying amounts. Complete profiles and essential oil yields of trunk, limb, needle, and cone samples are established. Published on-line www.phytologia.org *Published on-line www.phytologia.org Phytologia 102(3):200-207 (Sept 21, 2020). ISSN 030319430.*

KEY WORDS: *Pinus edulis,* Pinaceae, pinyon pine, essential oil, Utah, trunk, limbs, needles, cones.

Pinus edulis Engelm., also known as two-needle pinyon pine, is a small evergreen tree in the Pinaceae family (Flora of North America [FNA], 1993). *P. edulis* typically grows at elevations of 1500 – 2700 m and is often shrub-like, though it can grow to 21 m in height, with a wide branching crown; the bark is irregularly furrowed and scaly and there are two blue-green leaves per fascicle (Cronquist et al., 1972; FNA, 1993). It is native to Arizona, Colorado, New Mexico (with smaller populations in California, Wyoming, Oklahoma, and Texas), and Utah, where it often grows alongside *Juniperus osteosperma,* creating the pinyon/juniper forests that are common throughout the region (Cronquist et al., 1972; Ronco, 1990). It is monoecious, except under rare conditions, with most cones forming near the top of the crown (Ronco, 1990). Cones are pale yellow to pale red-brown, 3.5-5 cm, resinous, and mature over 2 years (Cronquist et al., 1972; FNA, 1993). *P. edulis* is a slow growing tree. It reaches full maturity between 75 and 200 years, and the tree can live up to 1000 years and produce seeds for centuries (Ronco, 1990). *P. edulis* has been found to hybridize with *P. monophylla* Torr. & Frém, also known as single-leaf pinyon, where species populations grow in close proximity (Lanner, 1974; Lanner and Phillips III, 1992).

Historically, pinyon pine has been an important tree among many Native American tribes for various reasons. The seeds, commonly called nuts because of their large size of 10-15mm, are an important food source due to their high nutritional value and ease of storage (Bentancourt, 1991; Schellbach, 1933). Schellbach (1933) documented many other practical and medicinal uses of *P. edulis:* the wood has been used for construction, tools, and firewood; the resin of the tree is useful as an adhesive, as well as in making containers waterproof; resin was often used as an antiseptic, protection for cuts and sores, or even to fill cavities; needles were eaten as a purported means to cure syphilis, and ulcers were treated with the powdered gum. Pencil shaped objects made using the gum were also used to extract and treat wounds from projectiles, such as arrows and bullets (Kindscher, 1992). Additionally, pinyon pine had great ceremonial importance in many communities (Schellbach, 1933).

Turpentine distilled from the oleoresin has been previously characterized (Snajberk, 1975; Mirov and Iloff, 1956) and shown to be primarily composed of α-pinene, δ-3-carene, and ethyl octanoate. Analysis of wood monoterpenes also showed high α-pinene, as well as δ-3-carene and limonene (Zavarin et al., 1989). The needle volatile emission, captured by dynamic headspace, has been characterized as being primarily composed of α-pinene, β-pinene, myrcene, and limonene (Trowbridge et al., 2019). To the authors knowledge, the steam distilled essential oil of the various plant parts has never been fully examined. In this study, the essential oil profiles of *Pinus edulis* seed cones, leaves, limbs, and trunk from Utah are established and compared. Distillation yields of the various plant parts are also reported.

MATERIALS AND METHODS

P. edulis plant material was collected with permission on private land in Tabiona, UT. Three trees in similar states of maturity were chosen and cut 10 cm above ground. Collection details are recorded in Table 1. Voucher samples are held in the Utah Valley University Herbarium (UVSC): *Pinus edulis* Engelm., Wilson 2020-01, -02, -03 (UVSC).

Three whole trees were harvested and separated into cones, needles, limbs, and trunk. Each separated portion was weighed. Cones, defined as the female or seed cone, and needles were distilled whole. Limbs, defined as leafless, 3-5 cm diameter sections nearest the trunk, were cut into segments 2-5 cm in length. The trunk, defined as heartwood, sapwood, cambium, and bark, was chipped. Enough material for three laboratory scale distillations of each plant portion from each tree, for a total of 36 samples from 3 trees (n=3), was retained and stored at -20°C until ready for distillation. All samples were steam distilled.

Laboratory scale distillation was as follows: 3 L of water added to the bottom of a 12 L distillation chamber (Albrigi Luigi S.R.L., Italy), plant material accurately weighed and added to the distillation chamber, distillation for 2 hours by direct steam, and essential oil separated by a cooled condenser and Florentine flask. Essential oil samples were filtered and stored in a sealed amber glass bottle in a cool, dark location until analysis.

Essential oils were analyzed, and volatile compounds identified, by GC/MS using an Agilent 7890B GC/5977B MSD and J&W DB-5, 0.25 mm x 60 m, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.1 µL of sample (neat essential oil, 0.1% soln. for C7-C30 alkanes in hexane), 150:1 split ratio, initial oven temperature of 40 °C with an initial hold time of 5 minutes, oven ramp rate of 4.5 °C per minute to 310 °C with a hold time of 5 minutes. The electron ionization energy was 70 eV, scan range 35–650 amu, scan rate 2.4 scans per second, source temperature 230 °C, and quadrupole temperature 150 °C. Volatile compounds were identified using the Adams volatile oil library (Adams, 2007, pdf at www.juniperus.org) using Chemstation library search in conjunction with retention indices. When identifications could not be made with the Adams library, the NIST Mass Spectral Library (version 2.3) was used and KI calculated using C7-C30 alkane standards. Note that limonene/β-phellandrene elute as a single peak, but their amounts are determined by the ratio of masses 68 and 79 (limonene), 77 and 93 (β-phellandrene). Volatile compounds were quantified and are reported as a relative area percent by GC/FID using an Agilent 7890B and J&W DB-5, 0.25 mm x 60 m, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.1 µL of sample (50% soln. for essential oils in ethanol, 10% soln. for reference compounds in ethanol, 0.1% soln. for C7-C30 alkanes in hexane), 25:1 split injection, initial oven temperature at 40 °C with an initial hold time of 2 minutes, oven ramp rate of 3.0 °C per minute to 250 °C with a hold time of 3 minutes. For quantification, compounds were identified using retention indices coupled with retention time data of reference compounds.

The percent yield was calculated as the ratio of mass of processed plant material immediately before distillation to the mass of essential oil produced, multiplied by 100.

RESULTS AND DISCUSSION

The aromatic profiles of trunk, limbs, needles, and cones from three *P. edulis* trees are detailed in Table 2. Each reported value is an average from three samples taken from that portion of each individual tree. Essential oil composition is similar in all, but relative percentages differ. Yield is included in Table 3. Cones averaged highest yield (0.7%) and needles averaged lowest (0.01%).

Essential oil obtained by steam distillation of the trunk of three *P. edulis* trees contained, respectively, primarily α-pinene (44.3%, 63.7%, 42.9%), ethyl octanoate (2.9%, 2.5%, 3.3%), and germacrene D (4.9%, 4.4%, 8.1%). Interestingly, the trunk essential oil of tree 1 and tree 3 contained high amounts of longifolene (9.6%, 10.4%) while tree 2 contained only a trace amount. Samples also showed variation in δ-3-carene, with tree 1 containing 10.0%, tree 2 containing 9.0%, and tree 3 containing only 2.9%.

Essential oil obtained from steam distillation of the limbs of three *P. edulis* trees contained, respectively, primarily α-pinene (57.8%, 64.3%, 56.9%), δ-3-carene (9.7%, 6.9%, 3.3%), ethyl octanoate (1.9%, 2.5%, 4.1%), and germacrene D (3.9%, 1.8%, 2.2%). In limb samples as well, longifolene was higher in tree 1 and tree 3 (3.7%, 4.8%), and only trace in tree 2.

Essential oil obtained from steam distillation of the needles of three *P. edulis* trees contained, respectively, primarily α-pinene (56.0%, 62.3%, 52.3%), β-pinene (2.6%, 1.7%, 7.1%), myrcene (3.1%, 1.2%, 1.8%), δ-3-carene (7.3%, 5.4%, 2.7%), beta phellandrene (6.7%, 2.9%, 2.0%), ethyl octanoate (1.7%, 1.7%, 2.7%) and bornyl acetate (0.8%, 3.1%, 4.5%). Consistent with trunk and limb samples, longifolene was lowest in the needles of tree 2.

Essential oil obtained from steam distillation of the cones of three *P. edulis* trees contained, respectively, primarily α-pinene (70.5%, 68.0%, 72.9%), sabinene (3.5%, 2.1%, 1.4%), β-pinene (3.2%, 2.3%, 3.4%), ethyl octanoate (0.9%, 1.3%, 2.6%), and β-bourbonene (0.9%, 1.6%, 2.3%). Longifolene was lowest in tree 2 for the cone samples as well. Cone essential oil of tree 1 and 2 contained high amounts of δ-3-carene (8.4%, 11.0%), while cones from tree 3 contained only 0.6%. It should be noted that the average cone weight for trees 1 and 2 was 6.1g and 8.7g, while average cone weight in tree 3 was 4.7g. Perhaps size of the cones could explain the difference in composition (Table 4).

CONCLUSION

This study confirms that *P. edulis* essential oil is of a similar composition to turpentine (Snajberk, 1975; Mirov and Iloff, 1956), the monoterpene analysis of the wood (Zavarin et al., 1989), and needle volatile emission (Trowbridge et al., 2019), with α-pinene, delta-3-carene, and ethyl octanoate being prominent compounds in the essential oils of all plant parts.

α-pinene is the prominent compound in every portion of *P. edulis,* with the highest amount present in the cones, then limbs, needles, and trunk. Longifolene is highest in the trunk and limbs, though this is not consistent in tree 2. Most cone essential oil samples were high in δ-3-carene, but not in the samples from tree 3. β-phellandrene is most prominent in the needles. Ethyl octanoate is present in similar amounts in essential oils from all sample types. Cones gave the highest yields and needles gave the lowest yields. These results show trends in the essential oil composition of the different parts of *P. edulis* but also that there is some variability despite trees being collected near the same location in similar states of maturity. Further research is needed to determine to cause of this variability.

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Table 1. Collection details for each individual tree.

	***Pinus edulis* #1**	***Pinus edulis* #2**	***Pinus edulis* #3**
date	3/10/2020	4/17/2020	5/1/2020
location	40°19'53" N 110°42'25" W	40°19'53" N 110°42'21" W	40°19'54" N 110°42'15" W
elevation (m)	2036	2036	2033
tree height (cm)	287	432	389
tree width (cm)	183	366	302
trunk weight (g)	13390	21050	20127
cone weight (g)	1420	1345	3036
needle weight (g)	24362	27636	28929
limb weight (g)	31343	29621	21266
total weight (g)	70515	79652	73358

Table 2. Aromatic profile of *P. edulis* essential oil from the trunk, limbs, needles, and cones of three pinyon trees. Each reported value below represents the average of 3 essential oil samples distilled from each portion (trunk, limbs, needles, cones) of the same tree. Compounds detected in one but not all samples are denoted as not detected (nd). Values less than 0.1% are denoted as traces (t). Unidentified compounds less than 0.5% are not included. KI is the Kovat's Index using a linear calculation on DB-5 column (Adams, 2007). Relative area percent is determined by GC-FID.
[1]Identified using the NIST Mass Spectral Library (version 2.3).
[2]Unidentified compound is suspected to be an isomer of ethyl octanoate. The KI was calculated using alkane standards. Prominent peaks in the mass spectrum include: 88 (100%), 101 (61%), 109 (33%), 55 (28%), 73 (25%).

		Pinyon Pine Averages											
		Trunk (%)			**Limbs (%)**			**Needles (%)**			**Cones (%)**		
KI	**Compound:**	1	2	3	1	2	3	1	2	3	1	2	3
921	tricyclene	0.1	0.2	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.2
924	α-thujene	0.1	t	0.2	0.1	t	0.1	0.1	0.0	0.0	0.1	0.1	0.1
932	α-pinene	44.3	63.7	42.9	57.8	64.3	56.9	56.0	62.3	52.3	70.5	68.0	72.9
945	α-fenchene	t	t	t	t	t	t	0.1	t	t	t	t	t
946	camphene	0.6	0.8	0.5	0.7	0.8	0.7	0.7	0.9	0.9	0.8	0.7	0.8
953	thuja-2,4(10) diene	0.3	0.3	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.3
969	sabinene	0.7	0.1	0.8	1.4	0.2	0.7	1.5	0.5	0.9	3.5	2.1	1.4
974	β-pinene	0.5	0.6	0.5	0.8	0.9	0.8	2.6	1.7	7.1	3.2	2.3	3.4
988	myrcene	0.8	0.9	0.6	1.5	1.0	1.0	3.1	1.2	1.8	0.7	1.4	0.6
1008	δ-3-carene	10.0	9.0	2.9	9.7	6.9	3.3	7.3	5.4	2.7	8.4	11.0	0.6
1014	α-terpinene	0.1	t	0.1	t	nd	nd	t	nd	nd	nd	nd	nd
1020	p-cymene	0.4	0.2	0.3	0.4	0.3	0.5	0.5	0.3	0.2	0.4	0.4	0.3
1024	limonene	2.0	0.6	0.5	2.2	0.6	0.5	3.3	0.5	0.4	3.8	0.7	0.8
1025	β-phellandrene	0.1	0.1	0.1	0.5	0.5	0.2	6.7	2.9	2.0	0.1	0.1	0.1
1026	1,8-cineole	nd	nd	nd	nd	nd	nd	t	t	nd	0.1	t	0.1
1032	(Z)-β-ocimene	0.3	0.5	1.5	0.3	0.2	0.5	0.2	0.2	0.1	0.1	0.1	0.2
1054	γ-terpinene	0.2	0.1	0.2	0.1	t	t	0.1	t	t	nd	nd	nd
[1]1068	methyl 6-methyl heptanoate (NIST 73%)	1.4	0.9	1.4	1.1	1.2	2.2	1.0	1.0	2.2	0.1	0.2	0.2
1086	terpinolene	0.1	t	0.1	0.2	0.3	0.3	0.1	0.3	0.5	0.4	0.4	0.5
1123	methyl octanoate	0.5	0.5	0.6	0.2	0.3	1.1	0.2	0.2	0.7	0.1	0.1	0.2
1122	α-campholenal	0.6	0.5	0.4	0.3	0.4	0.2	0.1	0.2	0.2	0.2	0.3	0.4
1135	trans-pinocarveol	0.6	0.3	0.3	0.2	0.2	0.2	0.1	0.2	0.2	0.4	0.6	0.6
[2]1154	unidentified compound	1.2	1.9	0.9	1.3	3.1	2.6	1.7	2.7	2.6	0.2	0.5	0.3
1158	trans-pinocamphone	0.2	0.1	0.1	0.1	0.1	0.0	nd	t	0.1	nd	nd	nd
1166	p-mentha-1,5-diene-8-ol	0.2	0.1	0.3	0.1	0.1	0.1	0.2	0.2	0.1	t	0.1	0.1
1174	terpinene-4-ol	0.1	0.1	0.1	0.2	0.2	0.2	0.4	0.4	0.5	0.1	0.2	0.1
1179	p-cymene-8-ol	0.1	0.1	0.1	t	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
1196	ethyl octanoate	2.9	2.5	3.3	1.9	2.5	4.1	1.7	1.7	2.7	0.9	1.3	2.6
1204	verbenone	0.3	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.2
1215	trans-carveol	0.1	0.1	0.1	0.1	0.1	t	t	t	t	t	t	0.1

KI	Compound:	Trunk (%)			Limbs (%)			Needles (%)			Cones (%)		
1241	methyl ether carvacrol	nd	t	t	0.1	0.1	t	0.3	0.4	0.4	nd	nd	nd
1287	bornyl acetate	0.7	0.7	0.5	0.4	0.7	0.6	0.8	3.1	4.5	0.3	0.5	0.6
1345	α-cubebene	0.4	0.1	0.5	0.3	0.2	0.4	0.3	0.4	t	0.1	t	0.2
1346	α-terpinyl acetate	0.1	0.1	0.2	0.1	0.1	0.1	0.4	0.2	t	t	0.2	0.1
1374	α-copaene	2.5	0.8	3.5	1.8	0.9	2.5	1.1	0.7	1.6	0.6	0.5	2.0
1380	ethyl-(4E)-decenoate	0.5	t	0.6	0.2	t	0.3	0.1	t	0.3	0.1	nd	0.1
1387	β-bourbonene	t	0.1	t	0.2	0.2	0.1	0.8	0.9	1.4	0.9	1.6	2.3
1389	β-elemene	0.2	0.3	0.3	0.1	0.3	0.3	0.2	0.2	0.2	0.1	0.1	0.2
1395	ethyl decanoate	0.2	0.4	0.5	0.1	0.3	0.4	0.1	0.2	0.3	0.1	0.1	0.2
1407	longifolene	9.6	t	10.4	3.7	t	4.8	1.2	0.1	1.6	0.9	t	2.0
1417	(E)-caryophyllene	0.5	0.5	0.8	0.4	0.3	0.4	0.2	0.3	0.3	0.1	0.2	0.4
1454	E-β-farnesene	0.5	0.5	0.5	0.3	0.4	0.3	0.1	0.2	0.1	nd	0.1	t
1465	(E)-ethyl cinnamate	0.1	0.1	0.2	0.1	nd	0.1	0.1	nd	0.1	nd	nd	0.1
1467	ethyl-(2E, 4Z)-decadienoate	0.4	t	0.7	0.1	t	0.2	0.0	t	0.1	t	t	t
1478	γ-muurolene	0.8	0.5	1.7	0.6	0.7	0.8	0.3	0.7	0.4	0.1	0.2	0.3
1484	germacrene D	4.9	4.4	8.1	3.9	1.8	2.2	0.7	1.2	0.8	0.0	0.2	0.2
1495	γ-amorphene	0.4	0.4	0.5	0.2	0.2	0.2	0.1	0.1	0.1	t	0.1	0.1
1500	α-muurolene	1.0	0.5	1.4	0.7	0.5	0.9	0.4	0.4	0.7	0.2	0.3	0.7
1513	γ-cadinene	0.6	0.4	1.0	0.7	0.9	0.6	0.5	0.9	0.4	0.1	0.1	0.2
1522	δ-cadinene	1.3	0.8	2.7	1.1	0.9	0.8	0.8	0.8	0.3	0.1	0.1	0.3

Table 3. Distribution of mass and essential oil (EO) yield averaged from samples from three *P. edulis* trees. Each tree was cut 10 cm above ground; all measurements and calculations are reflective of above ground portions.

		mass (g)	mass (%)	mass distilled (g)	yield EO (g)	yield EO (%)
Trunk	1	13390	19.0	897.0	3.58	0.4
	2	21050	26.4	1443.5	6.37	0.4
	3	20127	27.4	1295.1	4.93	0.4
	Avg:	18189	24.3	1211.9	4.96	0.4
Limbs	1	31334	44.4	3397.6	5.40	0.2
	2	29621	37.2	3254.9	4.75	0.1
	3	21266	29.0	3810.7	5.35	0.1
	Avg:	27407	36.9	3487.7	5.17	0.1
Needles	1	24362	34.5	1225.1	0.68	0.01
	2	27636	34.7	1434.7	1.24	0.01
	3	28929	39.4	1107.9	0.69	0.01
	Avg:	26976	36.2	1255.9	0.87	0.01
Cones	1	1420	2.0	469.9	3.43	0.7
	2	1345	1.7	445.0	3.01	0.7
	3	3036	4.1	958.7	4.25	0.4
	Avg:	1934	2.6	624.5	3.56	0.6

Table 4. Average weight and number of cones taken from the three pinyon trees.

	Cones		
	Tree 1	Tree 2	Tree 3
weight distilled (g)	469.9	445.0	958.7
# of cones	77	51	202
average cone weight (g)	6.1	8.7	4.7